# Use Of Single-Molecule Imaging To Analyze The Distribution Of Binding Ability In RNA Aptamer Populations

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**Use Of Single-Molecule Imaging To Analyze The Distribution Of Binding Ability In RNA Aptamer Populations**
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The number of possible sequences for nucleic acid and protein biopolymers of functionally plausible lengths is literally beyond astronomical, exceeding the probable number of atoms in the universe. The nature of the functionality landscape across sequence space for such molecules is of great interest to the origins of life field, and, more pragmatically, to those interested in the design, screening, or *in vitro* evolution of functionally useful molecules. This encompasses many questions relating characteristics of such molecules, such as stability or structural motifs, to the distribution of ability, as measured by kinetic rates. A key question concerns the likelihood of finding particular functional abilities (binding or catalysis) in a pool of sequences with a given length and/or other complexity-determining attribute.
Investigating the ''kinetic structure'' of a population is not possible with conventional bulk methods as subpopulations are simply averaged together. Such fine structure can only be approached using single-molecule techniques. This project uses a single-molecule fluorescence microscopy technique (Total Internal Reflection, or TIR) to analyze binding kinetics in populations of RNA aptamers. RNA is a particularly good candidate for exploration, owing both to its centrality in the RNA world hypothesis and the current interest in developing RNA aptamer based drugs.
Experiments with known GTP aptamers yield on and off rates that differ by species, are comparable to bulk results, and enable species separability in kinetic space. The goal is to enable quantification of the distribution of binding ability in heterogeneous, high complexity pools. In addition to addressing questions related to functional RNA, this is useful for designing and understanding *in vitro* selection experiments, a key tool for the origins of life field and the expanding field of applied molecular evolution.

**1479-Pos Board B323**
**Sensitivity Of Dna-hairpins Dynamics To The Mechanism Of Force Feedback Probed Using A Surface-coupled Passive Force Clamp**
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Optical-trapping experiments have yielded new insight into the mechanical behavior of individual biomolecules. A common experimental assay consists of an enzyme or nucleic acid molecule attached to a cover slip at one end and to a small polystyrene bead at the other. The bead is captured and held under tension with an optical trap. Active feedback maintains constant force, often called a force clamp, to increase measurement precision. Yet, active feedback is inherently bandwidth limited. This limited bandwidth leads to significant fluctuations in force that are particularly pronounced for rapid, large changes in molecule extension (e.g. DNA hairpin unfolding). A novel, passive force clamp circumvents this limitation by pulling the bead to a non-linear region of the trap where $k_{trap} = 0$. To date, this passive force clamp has required a specialized dual optical trapping apparatus where one trap measures position and the other measures force. Here, we demonstrate a passive force clamp achieved with a single trap in a surface-coupled assay using a previously characterized DNA hairpin. In an active force clamp, rapid back-and-forth transition between open and closed hairpins states were observed within the update period of the active force clamp (2 or 10 ms) as well as a change in the long term dynamics. By using a passive force clamp, these spurious transitions were eliminated and the correct dynamics measured. By analyzing the fluctuations in the bead position in conjunction with the known elasticity of DNA, we simultaneously measured force and position in a single-beam, passive force clamp. Thus, the benefits of the passive force clamp are now available to the widely used surface-coupled optical trapping assays.

**1480-Pos Board B324**
**Proof of Principle for Shotgun DNA Mapping by Unzipping**
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We are developing single-molecule methods for mapping protein-DNA interactions inside living cells by unzipping single chromatin fragments isolated from living cells. One avenue towards this capability involves unzipping random fragments that have been generated by site-specific restriction endonuclease digestion of whole genomic DNA or chromatin, a process we are calling shotgun DNA mapping or shotgun chromatin mapping. A key enabler of shotgun DNA mapping (SDM) will be the ability to assign the individual fragments to their specific sites in the genome, based on the sequence-dependent unzipping force of the underlying naked DNA sequence. We will present proof-of-principle results demonstrating the ability to match experimental data sets for pBR322 unzipping to the correct pBR322 sequence hidden in a library of approximately 3,000 yeast genome sequences arising from the known locations of XhoI recognition sites. We do so via an algorithm that scores the experimental data against simulated unzipping forces from a quasi-equilibrium model (Bockelmann, Essevaz-Roulet, & Heslot, 1997). Our next step is to perform SDM on yeast genomic DNA fragments produced by ligation of XhoI-digested DNA to unzipping constructs. Enhancements of the matching algorithm, data processing, and unzipping simulation will be discussed, along with studies of the robustness of the SDM method as a function of number of sites in genome and other parameters. In addition to the impact on our goal of single-molecule mapping of chromatin from living cells, SDM may have important applications in other areas of genomics, including high-throughput structural DNA mapping and genome-wide mapping of sequence-specific DNA binding proteins.

**1481-Pos Board B325**
**Direct Observation of DNA Untangling Magic by a Type-II DNA Topoisomerase**
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Type-II DNA topoisomerases(topoII) are ubiquitous enzymes that play key roles in the maintenance of DNA topology in cells. They control the degree of supercoiling of DNA and untangle the catenanes that arise during replication or recombination. Lack of their activities during cell division ultimately causes cell death. TopoII untangles DNA catenanes in an ATP-dependent manner, by catalyzing the transport of one DNA segment to the other side of a second DNA segment through a transient double-stranded break in the second segment. The work of topoII would seem like that of a magician who fascinates the audience, by solving a knot of rope without touching the knot.
Here, we show movies of this unlinking magic taken in real time under an optical microscope.

**1482-Pos Board B326**
**Real-time Observation of Positive Supercoiling by Reverse Gyrase**
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The degree of DNA supercoiling in living cells is an important factor that affects diverse processes such as DNA replication, transcription, and recombination. Supercoils are under control of DNA topoisomerases which either increase or decrease the linking number (Lk) of DNA. Type I topoisomerases cut one of the two strands of DNA, rotate and reseal the nicked strand, whereas type II cuts both strands to pass another DNA segment and then reseals. Although most of topoisomerases only relax DNA supercoils, DNA gyrase and reverse gyrase actively introduce negative and positive supercoils, respectively, into a substrate DNA. The reverse gyrase, found in thermophilic archea, is unusual because positive supercoils are normally harmful. Thermophiles are thought to exploit the reverse gyrase to overwind DNA to prevent denaturation at high temperatures. Bulk studies have shown that the reverse gyrase is an ATP-dependent type I topoisomerase active only at temperatures above 50°C. Here we show the action of reverse gyrase from *Sulfolobu*s in real time, tracking the rotation of DNA under a temperature-controlled microscope (50~70°C).

**1483-Pos Board B327**
**Effect of Single-Strand Break on Holliday Junction Migration Dynamics: A Single-Molecule Fluorescence Study**
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Single-strand breaks (SSBs) are the most common DNA lesions in living cells. SSBs occur three orders of magnitude more frequently than double-strand breaks. Unrepaired SSBs lead to blockage or collapse of DNA replication forks, possibly causing formation of double-strand breaks. Holliday junctions (HJ) play central role in various DNA functions including repair of lesions, replication, homologous and site-specific recombination. Branch migration, either spontaneous or protein-mediated, is among widely employed mechanisms in these functions. Therefore, elucidation of the SSB effect on spontaneous branch migration of HJs is a problem of great importance. To accomplish this task, we employed single-molecule FRET approach developed before, allowing us to follow spontaneous branch migration of one HJ at a single base pair level in real time. One SSB was incorporated in the middle of the homologous region of mobile HJ with the donor and acceptor dyes placed on its opposite arms. The data showed that branch migration does not stop at these lesions or reflects from them. Our previous results showed that branch migration is a step-wise process and one step can cover entire homology region. The analysis of the time trajectories showed that such